=> d his

(FILE 'HOME' ENTERED AT 10:25:49 ON 21 APR 2005)
FILE 'CA' ENTERED AT 10:25:59 ON 21 APR 2005
L1 2789 S NON?(5A)ENERGY TRANSFER?
L2 40 S L1 AND FRET
L3 66 S L1 AND(ENZYM? OR KINASE OR PHOSPHORYLASE OR TRANSAMINASE OR AMINASE OR CARBOXYLASE)
L4 10 S L1(8A)(SUBSTRATE OR ADDUCT)
L5 109 S L2-4
L6 60 S L5 NOT PY>1999
L7 5 S L5 NOT L6 AND PATENT/DT AND PY<2002
L8 44 S L5 NOT L6-7
L9 17 S L8 AND(REPORTER LABEL OR HOMOGENOUS OR NONRADIATIVE OR NON RADIATIVE)
L10 59 S L6 NOT(NONRADIOACT? OR NON RADIOACT?)
FILE 'BIOSIS' ENTERED AT 10:43:55 ON 21 APR 2005
L11 46 S L10
FILE 'MEDLINE' ENTERED AT 10:45:14 ON 21 APR 2005
L12 31 S L10
FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 10:46:29 ON 21 APR 2005
L13 105 DUP REM L7 L9 L10 L11 L12 (53 DUPLICATES REMOVED)

=> d bib,ab 1-105

L13 ANSWER 29 OF 105 CA COPYRIGHT 2005 ACS on STN
AN 127:342638 CA
TI Detection of probes in nucleic acid hybridization using **non-FRET** (fluorescence resonance **energy transfer**) pairs of chromophores
IN Tyagi, Sanjay; Kramer, Fred R.
PA Public Health Research Institute of the City of New York, Inc., USA; Tyagi, Sanjay; Kramer, Fred R.
SO PCT Int. Appl., 54 pp.
PI WO 9739008 A1 19971023 WO 1997-US6208 19970414
<--
US 6150097 A 20001121 US 1997-990176 19971212
<--
PRAI US 1996-15409P P 19960412
AB Nucleic acid hybridization probes is described having a first conformation when not interacting with a target and a second conformation when interacting with a target, and having the ability to bring a label pair into touching contact in one conformation and not the other, are labeled with a non-**FRET** pair of chromophores and generate a fluorescent or absorbance signal. As opposed to **FRET**, quenching mols. and even other fluorophores can serve as efficient quenching moieties for fluorophores when attached to nucleic acid hybridization probes such that the fluorescing moiety and quenching moiety are in contact, even when the rules of **FRET** are violated. To demonstrate probes with "touching" pairs of a fluorophore with another fluorophore or quencher, where the pairs are not **FRET** pairs, fluorescence quenching efficiency was measured where Mol. Beacon probes were end-labeled with DABCYL at one end and one of 8 different fluorophores at the other end. DABCYL could quench the fluorescence of fluorescein, Lucifer Yellow, BIDIPY, eosine, erythrosine, tetramethylrhodamine, Texas Red, and coumarin.

Effective fluorophore quenching also occurred in non-**FRET** pairs contg. other quenchers, DABMI and Malachite Green, as well as appropriate (shorter wavelength) fluorophores such as coumarin. The utility of fluorophore-quencher combinations is demonstrated in a multiplex detection assay using 4 different nucleic acid targets.

L13 ANSWER 33 OF 105 CA COPYRIGHT 2005 ACS on STN
AN 127:202400 CA
TI Fluorescence assays for DNA cleavage
AU Lee, S. Paul; Han, Myun K.
CS Department of Biochemistry, Georgetown University Medical Center, Washington, DC, 20007, USA
SO Methods in Enzymology (1997), 278(Fluorescence Spectroscopy), 343-363
AB Cleavage and joining reactions of nucleic acids are important processes in cellular events such as replication, recombination, and repair of DNA. Nucleic acids are readily cleaved by a variety of **enzymes** that recognize DNA sequences either specifically or nonspecifically. The efficiency of **enzymic** cleavage processes can be detd. by numerous methods such as gel electrophoresis, thin-layer chromatog., elution of the products from a DEAE-cellulose filter, and UV absorbance following HPLC to monitor disappearance of substrate or appearance of product. Current methods, though are either time-consuming or laborious. This work discusses the use of fluorescence spectroscopic approaches to design fluorescence assay systems monitoring **enzyme**-catalyzed DNA cleavage reactions. These assays are based on either **FRET** (fluorescence resonance **energy transfer**) or a **non-FRET** quenching mechanism.

L13 ANSWER 50 OF 105 CA COPYRIGHT 2005 ACS on STN
AN 121:225476 CA
TI A fluorometric assay for DNA cleavage reactions characterized with BamHI restriction endonuclease
AU Lee, S. Paul; Porter, Denise; Chirikjian, Jack G.; Knutson, Jay R.; Han, Myun K.
CS Dep. Biochem., Georgetown Univ. Med. Center, Washington, DC, 20007, USA
SO Analytical Biochemistry (1994), 220(2), 377-83
AB Fluorescently labeled oligonucleotides and DNA fragments have promise in nucleic acid research with applications that include DNA hybridization, automated DNA sequencing, fluorescence anisotropy, and resonance energy transfer studies. Past concerns with fluorescent-labeled DNA arose from interactions between fluorophores and DNA that result in quenched fluorescence. This quenching phenomenon is most problematic in fluorescence resonance energy transfer studies because quenching of the donor fluorescence could result from either resonance **energy transfer** or **nontransfer** effects. In the present study, relief of nontransfer quenching of a 14-mer fluorescein 5-isothiocyanate (FITC)-labeled oligonucleotide contg. the BamHI restriction site was characterized with both steady-state and time-resolved fluorescence techniques. The FITC-labeled single strand was best fit by a triexponential decay with lifetimes of 0.5, 2.7, and 4.2 ns. The 4.2-ns component was found to contribute more than 80% of the total steady-state intensity. Upon annealing with an unmodified complementary strand, the contribution from the 4.2-ns component was significantly decreased, resulting in twofold quenching of total fluorescence. The authors reasoned that this

quenching phenomenon should be a reversible process and could be employed to study strand sepn. processes in mol. biol. Hence, cleavage of the fluorescently labeled substrate was examd. using DNase I and BamHI restriction endonuclease. The authors' results show that the quenched fluorescence is totally recovered upon cleavage (compared to that of the single strand). The extent of cleavage measured by fluorescence was confirmed by nondenaturing polyacrylamide gel electrophoresis anal. The authors believe this fluorescence "dequenching" technique may be used to quantify the kinetics of other DNA strand sepn. and cleavage processes in mol. biol.

L13 ANSWER 51 OF 105 CA COPYRIGHT 2005 ACS on STN
AN 120:186037 CA
TI Resonance energy transfer determination of the distance between the four cysteine-364 residues in Saccharomyces cerevisiae phosphoenolpyruvate carboxykinase
AU Alvear, Marysol; Encinas, M. Victoria; Herrera, Lorena; Cardemil, Emilio
CS Fac. Ing. Adm., Univ. Frontera, Temuco, Chile
SO Archives of Biochemistry and Biophysics (1994), 309(2), 231-8
AB Each of the four subunits of the Saccharomyces cerevisiae phosphoenolpyruvate carboxykinase has one cysteine residue (Cys-364) that is protected against alkylation by MnATP and that is thought to be located at (or close to) the active site. To det. the distance relationships between these residues within this tetrameric **enzyme**, the authors have derivatized one of these reactive thiols with N-acetyl-N'-(5-sulfo-1-naphthyl)ethylenediamine (AEDANS) and the others progressively with 4-[N-[(acetoxy)ethyl]-N-methylamino]-7-nitrobenz-2-oxa-1,3-diazole (ANBD). In the doubly labeled protein **nonradiative** singlet-singlet **energy transfer** between AEDANS (donor) and ANBD (acceptor) was obsd. The efficiency of the energy transfer is proportional to the no. of occupied acceptor sites. From these data it has been detd. that one of the acceptor sites is 33 Å from the donor, and the remaining two sites are 44-46 Å from the donor. Crosslinking expts. revealed that mainly cross-linked dimers were produced upon reaction of the **enzyme** with o-phthalaldehyde and dithiobissuccinimidylpropionate. The authors interpret these results as an indication that this tetrameric **enzyme** is most likely composed of an assocn. of two dimers.

L13 ANSWER 62 OF 105 BIOSIS on STN
AN 1991:430633 BIOSIS
TI CONFORMATIONAL STUDIES OF A PEPTIDE CORRESPONDING TO A REGION OF THE CARBOXYL-TERMINUS OF RNASE A IMPLICATIONS AS A POTENTIAL CHAIN-FOLDING INITIATION SITE.
AU BEALS J M [Reprint author]; HAAS E; KRAUSZ S; SCHERAGA H A
CS BAKER LAB CHEM, CORNELL UNIV, ITHACA, NEW YORK 14853-1301, USA
SO Biochemistry, (1991) Vol. 30, No. 31, pp. 7680-7692.
AB Conformational properties of the OT-16 peptide, the C-terminal 20 amino acids of RNase A, were examined by noradiative energy transfer. A modified OT-16 peptide was prepared by solid-phase synthesis with the inclusion of diaminobutyric acid (DABA) at the C-terminus. The OT-16-DABA peptide was labeled with a fluorescent 1,5-dimethylaminonaphthalene sulfonyl (dansyl, DNS) acceptor at the N-terminal amine and a

fluorescent naphthoxyacetic acid (NAA) donor at the γ-amine of the DABA located at the C-terminus of the peptide by using an orthogonal protection scheme. Energy transfer was monitored in DNS-OT-16-DABA-NAA by using both fluorescence intensity (sensitized emission) and lifetime (donor quenching) experiments. The lifetime data indicate that the peptide system is a dynamic, flexible one. A detailed analysis, based on a dynamic model that includes a skewed Gaussian function to model the equilibrium distribution of interprobe distances and a mutual diffusion coefficient between the two probes to model conformational dynamics in the peptide [Beechem and Haas (1989) Biophys. J. 55, 1225.], identified the existence of a partially ordered structure (relatively narrow distribution of interprobe distances) at temperatures ≥ 20° C in the absence of denaturant. The width and the position of the average of the distributions decrease with increasing temperature, in this range; this suggests that the structure is stabilized by hydrophobic interactions. In addition, the peptide undergoes cold denaturation at around 1.5° C as indicated by broadening of the distance distribution. The addition of 6 M guanidine hydrochloride (Gdn-HCl) also broadens the distance distribution significantly, presumably by eliminating the hydrophobic interactions and unfolding the peptide. The results of the analysis of the distance distribution demonstrate that (1) **nonradiative energy transfer** can be used to study the conformational dynamics of peptides on the nanosecond time scale, (2) a partially ordered structure of OT-16-DABA exists in solution under typical refolding conditions, and (3) structural constraints (presumably hydrophobic interactions) necessary for the information of a chain-folding initiation site in RNase A are also present in the OT-16-DABA peptide in the absence of denaturant and are disrupted by Gdn-HCl.

L13 ANSWER 73 OF 105 BIOSIS on STN
AN 1987:291982 BIOSIS
TI ESTIMATION OF INTRAMOLECULAR DISTANCE DISTRIBUTIONS IN BOVINE PANCREATIC TRYPSIN INHIBITOR BY SITE-SPECIFIC LABELING AND **NONRADIATIVE** EXCITATION **ENERGY-TRANSFER** MEASUREMENTS.
AU AMIR D [Reprint author]; HAAS E
CS DEP LIFE SCIENCES, BAR-ILAN UNIV, RAMAT-GAN 52100, ISRAEL
SO Biochemistry, (1987) Vol. 26, No. 8, pp. 2162-2175.
AB A series of four bovine pancreatic trypsin inhibitor (BPTI) derivatives, site specifically labeled by (2-methoxy-1-naphthyl)methyl (MNA) at the N-terminal amino group and by [7-(dimethylamino)-coumarin-4-yl]acetyl (DA-coum) at one of the four ε-amino groups, was prepared. The four derivatives, Nα-MNA-Arg1-Nε-DA-coum-Lysn-BPTI [(1-n)BPTI] (n = 15, 26, 41, and 46), were purified by affinity chromatography and high-performance liquid chromatography (HPLC). The homogeneity of each derivative and its site of labeling were characterized by HPLC tryptic peptide mapping. **Nonradiative energy transfer** from MNA (donor) to DA-coum (acceptor) was measured by monitoring donor emission and acceptor excitation spectra. Transfer efficiencies between 45% and 85% were observed. The fluorescence decay of MNA in MNA-BPTI, a derivative labeled by a donor without an acceptor, is monoexponential, with a lifetime of 6.8 ± 0.15 ns. The decay kinetics of MNA fluorescence measured for derivatives labeled both by donor and acceptor showed a small deviation from monoexponential decay with shorter average

lifetimes. Analysis of the experimental decay curves yielded the detailed intramolecular distance distribution functions for each pair of labeled sites. The averages of the calculated distance distribution functions are close to the values expected from the known structure of BPTI in the crystalline state. The derivatives thus obtained are suitable for investigation of conformational transitions of the labeled protein and for monitoring localized changes such as those involved in the folding or unfolding transitions.

L13 ANSWER 76 OF 105 BIOSIS on STN
AN 1988:154811 BIOSIS
DN PREV198885078464; BA85:78464
TI AN APPARENT CONFORMATIONAL CHANGE IN PHENYLALANINE TRANSFER RNA THAT IS ASSOCIATED WITH THE PEPTIDYLTRANSFERASE REACTION.
AU ODOM O W [Reprint author]; HARDESTY B
CS CLAYTON FOUND BIOCHEM INST, DEP CHEM, UNIV TEX, AUSTIN, TEX 78712, USA
SO Biochimie (Paris), (1987) Vol. 69, No. 9, pp. 925-938.
AB Fluorescence techniques were used to detect changes in the conformation of tRNAPhe that may occur during the peptidyl transferase reaction in which the tRNA appears to move between binding sites on ribosomes. Such a conformational change may be a fundamental part of the translocation mechanism by which tRNA and mRNA are moved through ribosomes. Escherichia coli tRNAPhe was specifically labeled on acp3U47 and s4U8 or at the D positions 16 and 20. The labeled tRNAs were bound to ribosomes as deacylated tRNAPhe or AcPhe-tRNA. Changes in fluorescence quantum yield and anisotropy were measured upon binding to the ribosomes and during the peptidyl transferase reaction. In one set of experiments **non**-radiative **energy transfer** was measured between **a coumarin probe at position 16 or 20 and a fluorescein attached to acp3U47 on the same tRNAPhe molecule.** The results indicate that the apparent distance between the probes increases during deacylation of AcPhe-tRNA as a result of peptide bond formation. All of the results are consistent with but in themselves do not conclusively establish that tRNA undergoes a conformational change as well as movement during the peptidyl transferase reaction.

L13 ANSWER 101 OF 105 CA COPYRIGHT 2005 ACS on STN
AN 79:28720 CA
TI Use of substrates with fluorescent donor and acceptor chromophores for the kinetic assay of hydrolases
AU Carmel, Amos; Zur, Margalit; Yaron, Arieh; Katchalski, Ephraim
CS Dep. Biophys., Weizmann Inst. Sci., Rehovot, Israel
SO FEBS Letters (1973), 30(1), 11-14
AB A method is presented which is based on the interruption of **nonradiative energy transfer** between 2 chromophores attached to a **substrate** mol. **Enzymic** cleavage was followed, upon excitation of the donor, by monitoring either the increase in fluorescence of the donor or the decrease in the fluorescence of the acceptor. As an illustration of the method, the tryptic digestion of several peptides blocked by the donor 2-naphthylmethylamino and the acceptor 9-anthracenecarbonyl was studied.

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STN INTERNATIONAL LOGOFF AT 10:47:24 ON 21 APR 2005

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=> d his

     (FILE 'HOME' ENTERED AT 16:04:04 ON 20 APR 2005)
     FILE 'CA' ENTERED AT 16:04:28 ON 20 APR 2005
L1 291282 S KINASE OR PHOSPHORYLASE OR TRANSAMINASE OR AMINASE OR CARBOXYLASE
          OR CARBONYLASE
L2   7353 S L1 AND FLUORESCEN?
L3  66635 S L1(7A)(DETECT? OR DETERMIN? OR ASSAY? OR ANALY? OR TEST? OR
          MEASUR? OR MONITOR? OR ESTIMAT? OR INVESTGAT? OR STUDY? OR STUDIE#
          OR SCREEN? OR DISCOVER? OR EVALUAT? OR SENSE# OR SENSOR OR SENSING
          OR IDENTIF? OR PROBE# OR PROBING OR ASSESS? OR QUANTITAT? OR
          QUANTIF? OR ASCERTAIN? OR EXAMIN?)
L4   4364 S L1 (7A) INVESTIGAT?
L5   2351 S L2 AND L3-4
L6    335 S L5 AND(QUENCH? OR ENERGY TRANSFER?)
L7    159 S L6 NOT PY>1999
L8     82 S L6 NOT L7 AND PATENT/DT
L9    557 S L1 NOT L2 AND FLUOROMET?
L10   205 S L3-4 AND L9
L11     4 S L10 AND(QUENCH? OR ENERGY TRANSFER?)
L12     2 S L11 NOT PY>1999
L13     1 S L11 NOT L12 AND PATENT/DT
L14    26 S L8 AND PY<2002
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L15   105 S L7,L12
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L16    81 S L7,L12
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L17   210 DUP REM L7 L12 L13 L14 L15 L16 (164 DUPLICATES REMOVED)

=> d bib,ab 1-210

L17  ANSWER 15 OF 210  CA  COPYRIGHT 2005 ACS on STN
AN   133:331758  CA
TI   Optical probes and assays
IN   Pollok, Brian A.; Hamman, Brian D.; Rodems, Steven M.; Makings, Lewis R.
PA   Aurora Biosciences Corporation, USA
SO   PCT Int. Appl., 101 pp.
PI   WO 2000066766        A1    20001109    WO 2000-US12290        20000505
<--
     US 6410255           B1    20020625    US 1999-306542         19990505
PRAI US 1999-306542       A     19990505
AB   This invention provides an optical probe useful as an optical probe or
     sensor of post translational type modifications, such as
     phosphorylation.  The invention comprises a polypeptide moiety, which
     contains a recognition motif for a post translational type activity, and
     a protease site, which is coupled to a probe moiety.  Modification of
     the polypeptide, by the post translational type activity, results in a
     modulation of the rate at which a protease cleaves the polypeptide which
     is sensed by a measurable change in at least one optical property of the
     optical probe upon cleavage.  The present invention also includes a
     recombinant nucleic acid mol. that encodes an optical probe and a vector
     and host cell or library of cells that include the recombinant nucleic
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acid mol. The optical probe can be used in methods to det. whether a sample, including a cell or a sample from an organism, contains a post-translational type modification activity. Such methods can also be used to det. whether a test chem. modulates the activity of a modifying activity, and thus can be used to identify therapeutic compns. The identification of such therapeutic compns. can be automated using a system that includes an optical probe.

L17 ANSWER 50 OF 210 CA COPYRIGHT 2005 ACS on STN
AN 130:106745 CA
TI **Measurement** of cAMP-dependent protein **kinase** activity using a **fluorescent**-labeled Kemptide
AU Macala, Lawrence J.; Hayslett, John P.; Smallwood, Joan I.
CS Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA
SO Kidney International (1998), 54(5), 1746-1750
AB Traditional protein **kinase assays** include the use of 32P-labeled ATP as phosphate donor and a substrate protein or peptide as phosphoreceptor. Since this approach has a no. of drawbacks in addn. to generating ionizing radiation, several non-isotopic methods have been developed. Although shown to reflect the activity of purified enzymes, none have been demonstrated to detect physiol. changes in endogenous enzyme activity in cell homogenates. Here, studies were performed to examine the kinetics, reproducibility, and optimal **assay** conditions of a novel non-radioisotopic **kinase assay** that **detects** protein **kinase** A (PKA) activity by phosphorylation of the peptide substrate, Kemptide, covalently bound to a **fluorescent** mol. (fluorescamine-labeled Kemptide; f-Kemptide). **Fluorescence** was detd. by spectrofluorometry with excitation at 568 nm and emission at 592 nm. Basal and agonist-induced PKA activities in epithelial cell homogenates were measured. The kinetics of f-Kemptide were similar to the std. radioisotopic method with intra-assay and inter-assay variations of 5.6 ± 0.8% and 14.3 ± 2.6%, resp. Neither **fluorescence quenching** nor enhancing effects were found with consistent amts. of homogenate protein. Specific PKA activity was detd. as the IP20-inhibitable fraction to account for nonspecific phosphorylation, perhaps due to S6 **kinase** or a similar enzyme. The basal activity of 38% of total PKA in A6 cells increased by 84% after exposure to vasopressin and by 58% after short exposure to forskolin. In T84 cells exposed to VIP there was a 360% increase over basal activity. Thus, these results show that f-Kemptide exhibits acceptable kinetics, and that the assay system can quant. and reproducibly measure basal and stimulated PKA activity in cell homogenates.

L17 ANSWER 60 OF 210 CA COPYRIGHT 2005 ACS on STN
AN 127:231137 CA
TI Design and characterization of a multisite **fluorescence energy-transfer** system for protein folding studies: A steady-state and time-resolved **study** of yeast phosphoglycerate **kinase**
AU Lillo, M. Pilar; Beechem, Joseph M.; Szpikowska, Barbara K.; Sherman, Mark A.; Mas, Maria T.
CS Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, 37232, USA

SO Biochemistry (1997), 36(37), 11261-11272

AB A multisite distance-based **fluorescence** resonance **energy-transfer** assay system was developed for the study of protein folding reactions. Single- and double-cysteine substitution mutagenesis was utilized to place sulfhydryl residues throughout the tertiary structure of the bi-domain enzyme yeast phosphoglycerate **kinase** (PGK). These reactive cysteines were covalently modified with extrinsic donor [5-[[2-(2-iodoacetamido)ethyl]amino]-1-naphthalenesulfonic acid] and acceptor (5-iodoacetamidofluorescein) **fluorescent** labels. A detailed exptl. strategy was followed, which revealed that, when these relatively large extrinsic **fluorescent** labels are covalently attached to properly selected solvent-exposed residues, they do not affect the intrinsic stability of the protein. The PGK crystal structure was combined with mol. dynamics simulations of the dyes built into the protein and time-resolved anisotropy expts., in order to est. a more realistic orientation factor, $\langle\kappa 2\rangle^*$, for each donor/acceptor pair. Time-resolved and steady-state **fluorescence energy-transfer** expts. revealed that this distance assay, spanning six different donor-acceptor distances, is linear and accurate (to within 10-20%) over the range of 30-70 Å. This distance assay system for PGK allows for the measurement of long-range changes in intra- and interdomain spatial organization during protein folding reactions. The approach which we have developed can be applied to any protein system in which unique one- and two-site cysteine residues can be engineered into a protein. In the following paper [Lillo, M. P., et al. (1997) Biochem. 36, XXXXX-XXXXX], these multisite **energy-transfer** pairs are utilized for stopped-flow unfolding studies.

=> log y
STN INTERNATIONAL LOGOFF AT 16:39:27 ON 20 APR 2005